

## CLAIMS

We claim:

1. An array composition comprising:

- a) a substrate with a surface comprising discrete sites; and
  - b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent; and
  - c) at least one fiducial;
- wherein said microspheres are distributed on said surface.

2. An array composition according to claim 1 wherein each subpopulation comprises a unique optical signature.

3. An array composition according to claim 1 wherein each subpopulation comprises an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated.

4. An array composition according to claim 1 wherein said substrate is a fiber optic bundle and said fiducial is a fiducial fiber.

5. An array composition according to claim 1 wherein said substrate is a fiber optic bundle, said array comprises at least three non-linear fiducials, and each of said fiducials is a fiducial fiber.

6. An array composition according to claim 5 wherein at least one of said fiducial fibers has a different shape from the others.

7. An array composition according to claim 1 wherein said fiducial is a defined edge of said substrate.

8. An array composition according to claim 1 wherein said fiducial is a fiducial bead.

9. An array composition according to claim 1 wherein said bioactive agents are nucleic acids.

10. An array composition according to claim 1 wherein said bioactive agents are proteins.

11. An array composition according to claim 1 further comprising a computer readable memory comprising:

- a) computer code that receives a first data image; and
- b) computer code that registers said first data image using said fiducial to generate a first registered data image.

12. An array composition according to claim 11 wherein said computer readable memory further comprises:

- a) computer code that receives a second data image;
- b) computer code that registers said second data image using said fiducial to generate a second registered data image; and
- c) computer code that compares said first and said second data image.

13. A composition comprising a computer readable memory to direct a computer to function in a specified manner, said computer readable memory comprising:

- a) an acquisition module for receiving a data image of a random array comprising a plurality of discrete sites;
- b) a registration module for registering a data image; and
- c) a comparison module for comparing registered data images.

14. A composition according to claim 13 wherein said random array comprises a fiber optic bundle and said registration module utilizes a fiducial fiber for registration.

15. A composition according to claim 13 wherein said random array comprises microspheres and said registration module utilizes a fiducial microsphere for registration.

16. A composition according to claim 13 wherein said registration module utilizes a fiducial template for registration.

17. A composition according to claim 13 further comprising a random array comprising:

- a) a substrate with a surface comprising discrete sites; and
- b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent; wherein said microspheres are distributed on said surface.

18. A method of making an array composition comprising:

- a) forming a surface comprising individual sites on a substrate;
- b) distributing microspheres on said surface such that said individual sites contain microspheres, wherein said microspheres comprise at least a first and a second subpopulations each comprising a bioactive agent; and
- c) incorporating at least one fiducial onto said surface.

19. A method according to claim 18 wherein said subpopulations further comprise an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated.

20. A method according to claim 18 wherein said subpopulations further comprise an optical signature such that the identification of the bioactive agent can be elucidated.

21. A method according to claim 18 wherein said substrate is a fiber optic bundle and said fiducial is a fiducial fiber.

22. A method according to claim 18 wherein said substrate is a fiber optic bundle, said array comprises at least three non-linear fiducials, and each of said fiducials is a fiducial fiber.

23. A method according to claim 22 wherein at least one of said fiducial fibers has a different shape from the others.

24. A method according to claim 18 wherein said fiducial is a defined edge of said substrate.

25. A method according to claim 18 wherein said fiducial is a fiducial bead.

26. A method according to claim 18 wherein said bioactive agents are nucleic acids.

27. A method according to claim 18 wherein said bioactive agents are proteins.

28. A method for comparing separate data images of a random array comprising:  
a) using a computer system to register a first data image of said random array to produce a registered first data image;  
b) using said computer system to register a second data image of said random array to produce a registered second data image; and  
c) comparing said first and said second registered data image to determine any differences between them.

29. A method according to claim 28 wherein said random array comprises a fiber optic bundle and the registration of said first data image utilizes a fiducial fiber.

30. A method according to claim 28 wherein said random array comprises microspheres and the registration of said first data image utilizes a fiducial microsphere.

31. A method according to claim 28 wherein the the registration of said first data image utilizes a fiducial template.

32. A method of decoding a random array composition comprising

a) providing a random array composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent;

wherein said microspheres are distributed on said surface;

b) adding a first plurality of decoding binding ligands to said array composition and creating a first data image;

c) using a fiducial to generate a first registered data image;

d) adding a second plurality of decoding binding ligands to said array composition and creating a second data image;

e) using said fiducial to generate a second registered data image; and

f) using a computer system to compare said first and said second registered data image to identify the location of at least two bioactive agents.

33. A method according to claim 32 wherein said random array comprises a fiber optic bundle and the registration of said first data image utilizes a fiducial fiber.

34. A method according to claim 32 wherein said random array comprises microspheres and the registration of said first data image utilizes a fiducial microsphere.

35. A method according to claim 32 wherein the the registration of said first data image utilizes a fiducial template.

36. A method according to claim 32 wherein said bioactive agents are proteins.

37. A method according to claim 32 wherein said bioactive agents are nucleic acids.

38. A method of determining the presence of a target analyte in a sample comprising:

a) acquiring a first data image of a random array composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation each comprising a bioactive agent;

wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres;

b) registering said first data image to create a registered first data image;

- c) contacting said random array composition with said sample;
- d) acquiring a second data image from said array with said sample;
- e) registering said second data image to create a registered second data image; and
- f) comparing said first and said second registered data images to determine the presence or absence of said target analyte.

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39. A method according to claim 38 wherein said random array comprises a fiber optic bundle and the registration of said first data image utilizes a fiducial fiber.

40. A method according to claim 38 wherein said random array comprises microspheres and the registration of said first data image utilizes a fiducial microsphere.

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41. A method according to claim 38 wherein the the registration of said first data image utilizes a fiducial template.

42. A method according to claim 38 wherein said bioactive agents are proteins.

43. A method according to claim 38 wherein said bioactive agents are nucleic acids.

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